

The loss of pathogenicity associated with an inability to biosynthesize leucine has validated IPMDH as a potential fungicide target. This provides further evidence that biosynthetic enzymes may serve as targets for novel fungicides and has demonstrated that genetic target validation can identify new candidates for biochemical approaches to fungicide discovery.

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Involvement of the alternative oxidase in cellular energy production in the wheat 'take all' fungus, *Gaeumannomyces graminis* var *tritici*

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Abstract: This summary describes a functional role for the alternative respiratory pathway of the wheat 'take-all' fungus, *Gaeumannomyces graminis* var *tritici* (Ggt), which utilises the alternative oxidase as its terminal oxidase. Unlike other plant and fungal alternative oxidases, the alternative oxidase of Ggt is both constitutively expressed and active. We have demonstrated that for cellular respiration, and therefore for ATP synthesis, to occur both the cytochrome and alternative pathways must remain active.

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Keywords: *Gaeumannomyces graminis*; alternative respiration; ATP synthesis; electron transfer

Cellular energy production occurs in a tightly regulated manner with the complete oxidative metabolism of sugars producing carbon dioxide, water and ATP. Whilst this process normally yields between 32 and 38 ATP molecules per glucose molecule, only four of these ATPs are produced during glycolysis and within the TCA cycle. The remainder are formed by the mitochondrial ATP synthase, in a

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reaction driven by the energy liberated during transfer of electrons, through the electron-transfer chain, to oxygen as the terminal electron acceptor (Fig 1A). Transfer of the electrons from glucose metabolites to the initial complexes of the electron-transfer chain (complex I, complex II and/or via internal or external dehydrogenases) is by the co-enzymes, NADH and FADH₂. Reduction of complexes I, III and IV promotes proton translocation from the matrix to the inner mitochondrial space, which is in protonic equilibrium with the cytoplasm. This generates a pH gradient (ΔpH) and a membrane potential ($\Delta\Psi$) which together constitute the proton motive force (pmf) which provides energy for the synthase to generate ATP.

Until recently the scheme of electron flow through the electron-transport chain of filamentous fungi was assumed to be similar to that observed in mammalian and yeast mitochondria (Fig 1a). The sequential rise in potential (from negative to positive) of the components of the pathway ensures that electron flow is unidirectional from one redox centre to the next. In mammalian and yeast mitochondria there are at least ten different redox centres consisting of a series of cytochromes and iron-sulphur complexes. However, in many higher plant and some fungal mitochondria, the components and sequence of events appear more diverse, involving alternative redox centres and pathways which diverge from the cytochrome-based system (for review see Ref 1).

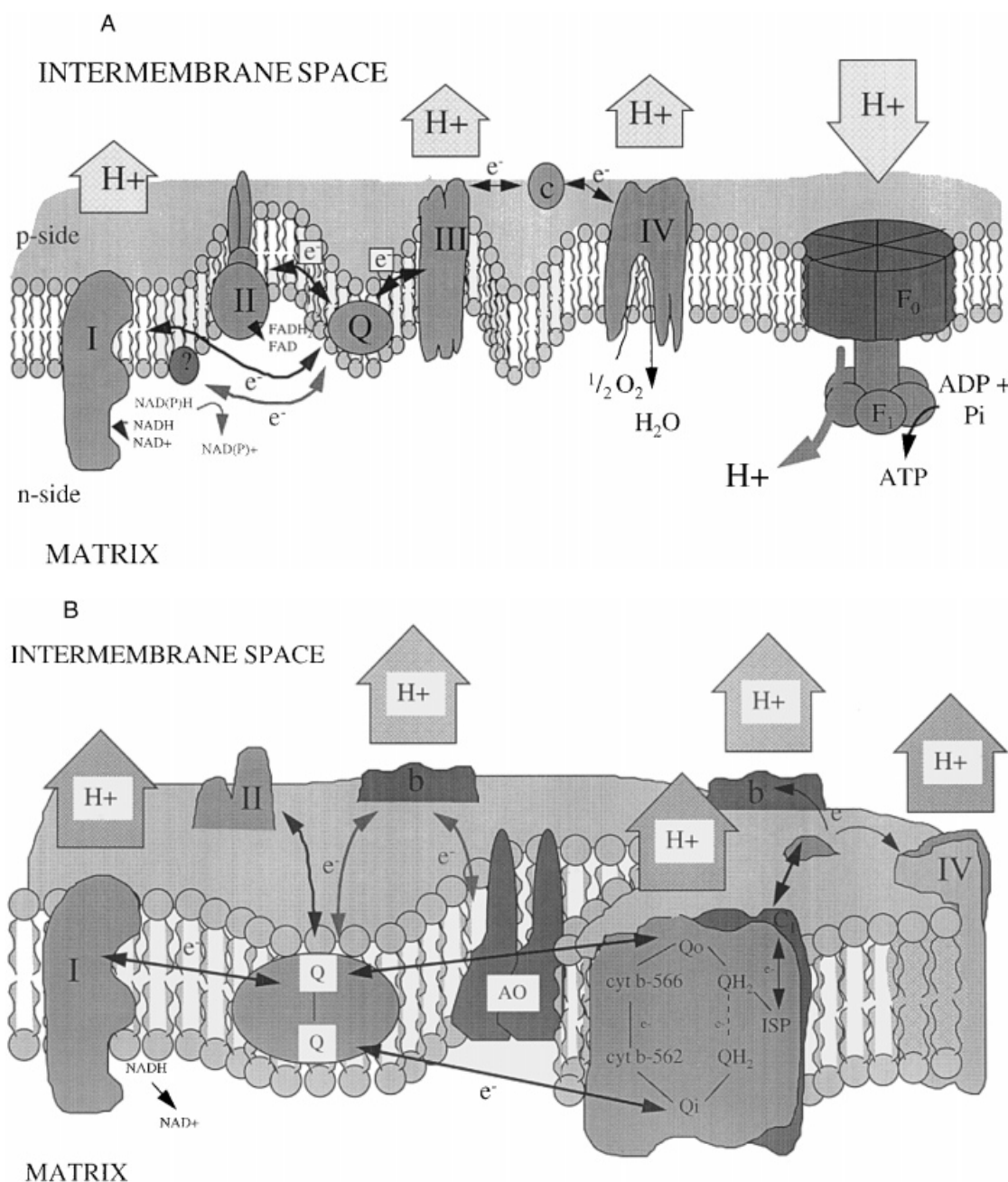


Figure 1. Schematic representation of the electron transfer chain of A: mammalian mitochondria, and B: the proposed electron transfer chain of Ggt. Arrows indicate the direction of electron flow between the redox centres and sites of H⁺ translocation. I, Complex I; Complex II; III, Complex III; IV, Complex IV; ISP, iron-sulphur protein; Q, quinone:quinol pool; AO, alternative oxidase and b, b-type cytochrome.

The requirement of the respiratory chain for all aerobic cells has presented the agrochemical and pharmaceutical industries with a powerful potential target. However, the strongly conserved nature of the components involved has ensured that most compounds with activity within this system suffer from adverse toxicological profiles (eg cyanide). To date only carboxanilides, rotenone and, more recently, the methoxyacrylate group of chemicals, have displayed sufficient specificity to enable their launch as agrochemicals.

The development of such compounds has renewed interest in fungal respiration. Unlike the pathway described in Fig 1A, the scope for diversity appears greater in fungi than has been previously anticipated. We have demonstrated that in the wheat 'take-all' fungus, *Gaeumannomyces graminis* (Sacc) Arx & Oliver var *tritici* Walker (Ggt), a second respiratory pathway is present and constitutively active (Fig 1B).² Three techniques provided evidence that this pathway ends in a terminal oxidase: whole-cell sensitivity to salicylhydroxamic acid (SHAM) (assessed by growth inhibition and inhibition of oxygen consumption); decline of membrane potential in isolated mitochondria; and cross-reaction of mitochondrial proteins with the plant alternative oxidase antibody. Many of the data on which these statements are bound have been published elsewhere, but the involvement of this alternative respiratory chain in ATP production will now be examined more fully.

The ability of whole Ggt cells to synthesize ATP after the selective inhibition of specific components of the respiratory chain was assessed over 15 min, using the inhibitors oligomycin B, antimycin A, SHAM and combined antimycin A + SHAM (Fig

2). In agreement with previous studies, ATP levels in untreated cells remained constant over the assay period. As expected, there was a strong reduction in ATP levels following complex III inhibition by antimycin A, to a quarter of the basal level by the end of the 15-min treatment. However, inhibition of the alternative oxidase by SHAM decreased ATP levels by 58% over an identical time period, providing evidence that the alternative pathway has an essential function in ATP generation. Further evidence in support of this conclusion was obtained from a combined treatment study with SHAM + antimycin A, which caused a more rapid inhibition of cellular ATP levels than either treatment alone. The experiment led to a 77% inhibition after 15 min, which is similar to the 80% inhibition observed after treatment with oligomycin B (an inhibitor of the ATP synthase). These data indicate that electron flow along an alternative pathway with a SHAM sensitive terminal oxidase leads to mitochondrial ATP synthesis. This implies a coupling of electron flow to proton translocation. Supporting evidence was provided by monitoring a change in membrane potential on addition of SHAM to isolated mitochondria, as described elsewhere.²

We have shown that the alternative oxidase of Ggt is expressed constitutively in an active dimeric form.² This is in contrast to a relatively small number of other fungi, namely *Hansenula anomala* Syd,³ *Botrytis cinerea* Pers ex Fr⁴ and *Magnaporthe grisea* (Hebert) Barr,⁵ in which it was only activated after saturation or inhibition of the cytochrome pathway.

In plants, where electron flow to the alternative oxidase is not coupled to proton translocation, and

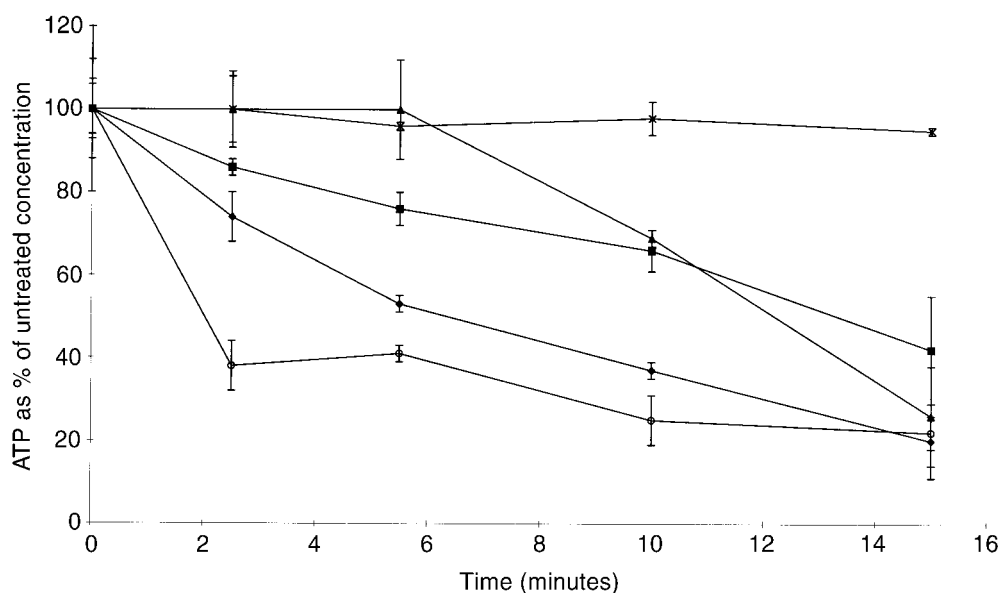


Figure 2. Effect of inhibitors on ATP levels in whole Ggt cells. ATP levels were assessed (X) in the absence of inhibitors and following treatment with (◆) oligomycin (7 μ M), (▲) antimycin A (20 μ M), (■) SHAM (0.3 mM), (○) combined antimycin A + SHAM (20 μ M + 0.3 mM). ATP was extracted into phenol:chloroform (1:1) from mycelia which had been ground after flash freezing as described in Ref 2. ATP quantification of the resultant aqueous phase employed the coupled luciferase:luciferin assay (Molecular Probes Inc, Europe) with the bioluminescence recorded referenced to a standard ATP concentration curve. All standard deviations were calculated from duplicated assays with triplicated sampling.

hence does not contribute to ATP synthesis, the function of the pathway has been clarified in recent years. It is expressed and activated during periods of stress, for example in response to wounding or flowering.¹ This activation provides plant mitochondria with a mechanism by which an increased rate of respiration can be achieved. There is no evidence that Ggt responds to stress in a similar way, so its alternative pathway must have a different function.

One explanation for the presence of the alternative oxidase in Ggt is that its K_m for oxygen is lower than that of cytochrome oxidase for oxygen. This would suggest that the constitutive expression of the alternative oxidase could be an adaptive response to low oxygen concentrations often associated with the environment surrounding plant root systems.

Overall this study has demonstrated that variation in the components of the electron-transfer chain within the mitochondria of filamentous fungi is possible (Fig 1B). It also implicates involvement of novel complexes in the process of electron transfer, and ultimately in providing cellular ATP. With the development and launch of novel chemistries targeting energy production, such as the methoxyacrylates, the requirement to characterise fully fungal respiratory chains is essential in order to adopt suitable resistant management strategies and for the design of novel chemistries.

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Synthesis and herbicidal activity of pyrimidine derivatives

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Abstract: A new class of 2-benzoylpyrimidines exhibited good pre- and post-emergence herbicidal activity at low rates. The selectivity and herbicidal activity were largely affected by the substituents on the phenyl and pyrimidine moieties. The compound having a trifluoromethyl group at the 4-position of the pyrimidine showed good selectivity toward soybean in pre-emergence treatment, while the compound having an isopropyl group showed excellent herbicidal activity against barnyardgrass in paddy field in post-emergence treatment. The structure-activity relationship and mode of action of this family of compounds are discussed.

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Keywords: benzoylpyrimidine; herbicidal activity; selectivity; cell division inhibition

A number of pyrimidine derivatives are known to be bioactive, the pyrimidine substructures often being found in well-known pesticides such as herbicidal sulfonylureas and pyrimidinylsalicylic acids, and in fungicidal anilinopyrimidines. The two types of pyrimidine herbicide eg sulfometuron-methyl and pyriminobac-methyl, both inhibit acetolactate synthase (ALS) in spite of the structural differences in the bridge between the pyrimidine and benzene rings, ie 'sulfonylurea' in sulfometuron-methyl and 'ether' in pyriminobac-methyl. The sulfide analogues of the ether compounds such as pyrithiobac-sodium are also known as potent ALS-inhibiting herbicides. On the other hand, the anilinopyrimidines, such as mepanipyrim, having an amino bridge between the 2-position of pyrimidine and a phenyl group, show fungicidal activity. We were interested in the role of the bridge moiety in biological activity, and a survey of the literature and patents of the related compounds revealed that there had been few reports on the synthesis of 2-benzoylpyrimidines (1; Table 1) having a carbonyl group at the bridge moiety.^{1–3} We have accordingly investigated the synthesis of compounds of type 1 as new candidate pesticides, since their biological properties were unknown.

The 2-benzoylpyrimidines (1) were synthesized by the oxidation of the corresponding benzylpyrimidines, which were obtained from the appropriate benzylamidines and β -ketoesters or β -diketones (Fig. 1). Among a series of the benzoylpyrimidines, the compounds having a trifluoromethyl or an isopropyl group at the 4-position (R_1) of the pyrimidine ring exhibited potent activity against weed species and selectivity toward soybean in pre-emergence treatment. Investigation of the effects of the benzene-ring substituents revealed that the 2- or 2, 6-substituted compounds, such as 2-chloro, showed a potent activity. On the other hand, the 2, 3- or 2, 4-disubstituted compounds were less active, and the

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